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STUDIES ON THE KARYOMORPHOLOGICAL CONSEQUENCES OF MALEIC HYDRAZIDE ON DIFFERENT VARIETIES OF *Trigonella foenum- graceum* L.

Submitted by:

JYOTHSANA BESRA

Under the supervision of

Dr. Neetu Rani

Assistant professor

Nirmala college, Ranchi

Ranchi University, Ranchi- 834008

Jharkhand

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STATE OF ART

"Fenugreek (*Trigonella foenum-graecum L.*) own an important place in the realm of spice world". In addition to its culinary applications, Fenugreek is thought to be the oldest known medicinal herb in history[18]. Belonging to Family Fabaceae, Fenugreek is an aromatic herb that grows every year with 2n=16 chromosome number. It is considered as multipurpose herb of short duration. It has long been believed to be a medicinal plant and is distinctly used. India currently accounts for over 68% of global production, making it the world's leading producing nation[18]. Since primitive time, Fenugreek is regarded as safe for human health and that medical science has undeniably recognized its nutritional value and biological active compound profile[18]. It has a high protein content. The medicinal benefits of Fenugreek seeds include promoting lactation, acting as an antibacterial agent and gastric stimulant, lowering blood cholesterol, treating anorexia, galactagogue, having a hepatoprotective effect and anticancer properties[19]. The fiber, gum, other chemical components and volatile content of fenugreek are well known. Numerous coumarin compounds and alkaloids including trigonelline, carpaine, diosgenin, methionine, saponin, gentianine and others have been discovered to be present in its seeds[19].

Protein is one of the important macronutrients which play a vital role in muscle building, skin, wound healing and for growth. Diabetic people are more prone to muscle loss, foot ulcer and weight loss. So, intake of protein is important for diabetes management and also helpful in glycemic control.

A prevalent technique for increasing variability for crop improvement is induced mutagenesis. Mutation breeding is one of the most effective way to induce mutation for the efficient mutant selection in self-pollinating crop Fenugreek for better yield quality and growth habit[18]. In recent years, mutation breeding has gained popularity as a useful technique for crop improvement[10]. Plant evolution and emergence of new species are significantly influenced by mutation. "The role of mutation in evolution was emphasized by Baur (1924). Mutation breeding attracted considerable attention during 1950s and 1960s and several countries took up research project in Mutation breeding"[8]. A technique to overcome the constraints of plant variability and make targeted improvement in crop varieties without compromising their superior quality is the artificial induction of mutation[8]. The majority of mutagen are biologically

active and can cause biological reactions that show up at the molecular, cellular, organismic population levels as well as ecosystem level[5]. The majority of mutagens are physiologically active and can cause biological reactions brought on by herbicide-induced adaptive responses has sparked a lot of interest recently since living organisms, including plants under mutagen.[5].

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) is a powerful herbicide that inhibits plant growth and has been used in agriculture[10]. It is used to reduce dormancy in some crops and delay the development of buds, extending the shelf life of vegetables and ensuring to reach the consumer fresher. It is a pyridazine that prevent the synthesis of nucleic acid and proteins[10]. It is a well-known genotoxic agent in plants that causes sister chromatid exchanges and chromosomal abnormalities in plants[10].Given the widespread use of herbicides in agriculture and their possible carcinogenicity, it is imperative that these compounds be further evaluated using a variety of techniques[10].

There are few reports on the "Effects of maleic hydrazide on root tip cells of *Trigonella foenum- graceum*". Some of them are: Earlier it was reported on "Maleic hydrazide (MH) induced variations for economic traits in *Trigonella foenum- graecum* L". The studies came to conclusion that increased mutations and recombination may be the cause of high variability in polygenic traits. Therefore, the desired changes in the Fenugreek genotype can be effectively induced through the induction of variability to obtain better variety[8]. Further studies on the "Effect of Maleic hydrazide on root tip cells and pollen fertility in *Trigonella foenum-graceum* L". was also reported. This study concluded that MH has a toxic effect on Fenugreek spore mother cells, root tip cells and vegetative cells[10].

It was reported that "Glyphosate, Alachor and Maleic hydrazide have genotoxic effect on *Trigonella foenum- graecum* L." By these studies some abnormalities were observed such as unequal distribution and fragments, laggards, bridges, stickiness, c-anaphase and precocious separation. According to the investigation's findings, the Fenugreek plant is significantly genotoxically affected by the widely used herbicides GP, AL and MH[13]. "Cytological studies in the Genus *Phaseolus* I. Mitotic analysis in fourteen species" was reported from this work they conclude that karyotype asymmetry and change in the morphology of the chromosomes were instrumental in speciation within the genus *Phaseolus*[2]. "Cytotaxonomy of *Trigonella foenum graceum* L. collected from Jharkhand and Uttar Pradesh" was also reported[14]. "Effect of some

common herbicide on root tip cells of *Trigonella foenum- graceum* was reported"[24].

Work on "Maleic hydrazide induced variability in Fenugreek (*Trigonella foenum-graecum* L.) cultivars CO1 and Rmt-1" was also reported. The experiment elucidated that the induced variation with 0.06% could be used for new cultivar generation in Fenugreek. Fenugreek cultivars with increase yield, early maturity and fast growth could be created using this mutation breeding technique[18]. Furthermore, studies on "Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L". This investigation shows nucleolar changes that point to biosynthetic activity inhibitory effect[7]. It was also reported that "The influence of maleic hydrazide preharvest treatment on quality and storage ability of early onion cultivars at cold store conditions". The outcome demonstrated that MH treatment significantly reduced the storage losses of early-maturing onion cultivars[11].

"Influence of dietary protein on postprandial blood glucose levels in individuals with Type 1 diabetes mellitus using intensive insulin therapy". This backs up suggestions for insulin dosage for protein intake[17].

Few works on the "Effect of maleic hydrazide on root tip cell of *Trigonella foenum- graecum* L". has been undertaken but till date no work on the fenugreek varieties Afg-3, Afg-4, Afg-5 and estimation of the protein content have been done. With a larger rate of diversity in qualitative and quantitative qualities, the current study attempts to fill the indicated gap in terms of nutritional value, medicinal benefit and fewer biological harms.

PROBLEM TO BE INVESTIGATED

The Karyomorphological consequences of Maleic Hydrazide on different varieties of *Trigonella foenum- graceum* L. for its promotion as

- To estimate the genotoxic effect of MH through mitosis.
- By the advancement of mutational approach, can obtain the desired trait in commercial cultivar with high yield or not?
- Is there any possibility of changes in plant attribute inducing herbicides on the variety?
- Post treatment protein estimation in *Trigonella* seeds.

OBJECTIVE OF INVESTIGATION AND MOTIVATION

The purposed work "**THE KARYOMORPHOLOGICAL CONSEQUENCES OF MALEIC HYDRAZIDE ON DIFFERENT VARIETIES OF *Trigonella foenum- graceum* L.**" has been undertaken with the following objectives: -

- Karyotype analysis
- Studies on Induced variations following maleic hydrazide treatment.
- Studies on qualitative mutations of mutant.
- Quantitative estimation of seed protein
- Stomatal studies

MOTIVATION

"Trigonella is regarded as the oldest known medicinal plant in recorded history (Lust 1986)". Because Fenugreek seeds and leaves have numerous medicinal benefits, they have been used to lower blood cholesterol and blood sugar levels in people. It is also considered as a multipurpose cash crop of short duration. So, it is important to find the ways to improve its qualitative and quantitative characters. Therefore, the main motivation of the present research work is:

- To create new mutant lines with better economic traits and improve the genetic variety of plants
- Estimating protein content

HYPOTHESIS

The studies on the Karyomorphological consequences of maleic hydrazide on different varieties of *Trigonella foenum-graceum* L. can result the chromosome aberration induction. "As Khilman (1956) reported that the effect of maleic hydrazide on *Vicia faba* chromosomes was largely dependent on the concentration of the treatment solution, higher the concentration of herbicides, higher the yield of chromatid aberrations".

As the *Trigonella* is mainly used as a medicinal plant, through the studies by inducing maleic hydrazide on *Trigonella* there is increment or decrement in their protein content.

RESEARCH DESIGN & METHODOLOGY

Methodology will be only laboratory-based work.

Laboratory-based work: -

i. Sample collection-

Three varieties of *Trigonella* were procured from ICAR- National Research Centre of seed spices, Ajmer.

ii. Karyotype Analysis

- Mean length
- Relative length
- F%
- Total Form%
- TCL

Squash technique is used for karyotype analysis. Following methods are used in squash technique.

1. Placing a root tip on watch glass, a few drop of 1% acetocarmine were added and the root was left to stain for 20 -30 minutes.

2. After cutting the distal end of the stain root tip, it was put on a glass slide in 45% acetic acid and left for 2 minutes.

3. After placing the cover slip over the stained root tip, the cover slip was kept hidden beneath the blotting paper folds as the root tip was compressed with tapper.

4. The coverslip edgers were sealed with wax or nail polish.

5. The slide was observed under a microscope.

iii. Meiotic Study: To find out the chiasma frequency, variance ratio and correlation coefficient among different varieties of *Trigonella foenum-graceum.*

iv. Studies on induced variations following maleic hydrazide treatment:

- Raising of M1 plant population
- Assessment of seed germination and seedling length
- Studies of Mitotic aberration

- Study on pollen fertility
- Efficiency and effectiveness of the mutagen

Preparation of chemical (MH)- Different concentration of MH is prepared from stock solution.

v. **Qualitative analysis: -**
Different morphological attributes like mean number of secondary branches, plant height, mean no. of flowers and pods per plant will be done after treatment with MH.

vi. **Quantitative analysis: -**
Post treatment protein estimation in M1 and M2 population of seeds will be done by Lowry method (1951).

vii. **Stomatal studies**
Stomatal index can be calculated by the formula: -

$$\text{S.I.} = \frac{S}{E+S} \times 100$$

MATERIALS TO BE USED IN THE COLLECTION OF DATA

Sample required –

Three varieties of *Trigonella foenum-graceum* seeds- Afg-3, Afg-4, Afg-5

Apparatus required –

Slide, watch glass, coverslip, dropper, blade, needle, burner, forceps, measuring cylinder, brush, burner stand, burner mat, sample container, blotting paper, tapper, pipettes, test tube

Various chemical required-

Acetocarmine, para-dichlorobenzene, acetic acid, maleic hydrazide, distilled water, ethanol, copper sulphate, copper reagent, sodium hydroxide, sodium carbonate

Machine required

Microscope, weighing machine, Refrigerator, colorimeter, spectrophotometer

Place of work

Work is mostly laboratory based and thus will be done in Nirmala college, Department of Botany, Ranchi.

SIGNIFICANCE OF THE STUDY

The present study is for reaching significance-

1. To study the genotoxicity of MH on chromosome.
2. To evaluate improved economic traits which would be useful in plant improvement and breeding
3. Post treatment estimation of protein content in *Trigonella*

IT'S RELEVANCE TO PRESENT DAY PROBLEMS AND NEED OF THE SOCIETY

India is the second largest country having diabetic patients. Diabetes is a terrible illness, is brought on by either the pancreas producing insufficient amount of insulin or our bodies not using it efficiently. Diabetes causes foot ulcer, kidney failure, retinal damage, muscle loss, risk of heart disease etc.

"According to WHO, In India there are 77 million people above the age of 18 years are suffering from type 2 diabetes and nearly 25 million are prediabetics. It is expected to rise in the number of people with diabetes over 124 million by 2045 (SRD, 2023)". *Trigonella* have ameliorative and hypoglycemic effects on diabetes. Protein is essential for building muscle, so intake of protein is essential for diabetic patient.

ECONOMIC REVIVAL

1. Maleic hydrazide accelerates evolution by inducing various genic and chromosomal variants, increasing the rate of morphological and yield character variations for the selection of superior trait.
2. Economic revival of genotoxicity studies is analyzing increment or decrement in protein content of *Trigonella.*

CONTRIBUTION TO THE EXISTING KNOWLEDGE

1. Induced mutagenesis by genotoxicity effect of maleic hydrazide on *Trigonell*a can be widely used for increasing crop variability that can be extensively used.
2. The current research work will assist in examining in the extent of variations in qualitative and quantitative traits in Fenugreek.
3. Quantitative estimation of protein content is necessary so as to observe the increment in their medicinal value and used as an antidiabetic source.

FUTURE WORK

Genetic analysis of *Trigonella* germplasm by molecular marker will help in the understanding extent of genetic diversity and varietal identification, to obtain more reliable results for the pharmaceutical industry can be achieve in future work.

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